

PCR gld (Fasl mutant)
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2 separate PCR are needed :

1- **wild type** PCR mix

2,50 µl 10X buffer(Q BIOgene ; 1,5 mM MgCl<sub>2</sub>)  
 0,50 µl dNTP (20 mM total)  
 0,08 µl primer Fasl-c (200 ng/µl)  
 0,80 µl primer Fasl-s (200 ng/µl)  
 0,40 µl primer Fasl-rev (200 ng/µl)  
 0,20 µl Taq polymerase (Q BIOgene ; 5U/µl)  
 19,52 µl distilled water

2- **mutant** PCR mix

2,50 µl 10X buffer(Q BIOgene ; 1,5 mM MgCl<sub>2</sub>)  
 0,50 µl dNTP (20 mM total)  
 0,08 µl primer Fasl-c (200 ng/µl)  
 0,80 µl primer Fasl-gld (200 ng/µl)  
 0,40 µl primer Fasl-rev (200 ng/µl)  
 0,20 µl Taq polymerase (Q BIOgene ; 5U/µl)  
 19,52 µl distilled water

3- PCR reaction :

24,0 µl mix  
 1,0 µl DNA (200ng/µl)

4- PCR program :

5 min	94°C	
30 sec	94°C	
40 sec	60°C	40 cycles
1 min	72°C	
5 min	72°C	

5- Primers:

- Primer Fasl-c                    5' GAAGTATAAGAAAGGTGGCC 3'  
 - Primer Fasl-s                    5' CAATTTTGAGGAATCTAAGACAT 3'  
 - Primer Fasl-gld                5' CAATTTTGAGGAATCTAAGACA 3'  
 - Primer Fasl-rev                5' GTAAAGATGGTGCCAATGAG 3'

6- Amplifications:

**wild type** PCR mix : control allele : 816 bp    wild type allele :536 bp

**mutant** PCR mix : control allele : 816 bp    mutant allele : 536 bp